

AMENDMENTS TO THE SPECIFICATION:

Please amend the Specification as follows:

Kindly replace the paragraph beginning at page 2, line 20, with the following:

As the peptide of interest, for example, there can be mentioned human glucagon-like peptide-1 ("GLP-1") ~~(referred to hereinafter as GLP-1;~~ (Bell GI et al., Nature, Vol. 304, pp. 368-371, 1983) and derivatives of GLP-1 (referred to hereinafter as GLP-1 derivatives) having an insulintropic activity. GLP-1 is a peptide consisting of 37 amino acid residues derived from proglucagon. Through processing of proglucagon, GLP-1(7-37) in which 6 amino acids at the N-terminal of GLP-1 are deleted and GLP-1(7-36)NH₂ in which the C-terminal of GLP-1(7-36) has been modified to an amide form have been biosynthesized (Mojsow, S. et al., J. Clin. Invest. Vol. 79, pp. 616-619, 1987). Since these peptide hormones have an activity of promoting the secretion of insulin by acting on the beta cells in the pancreas, the possibility as candidates of a therapeutic agent for diabetics has been proposed based on their pharmacological effects in recent years (Gutniak M. K., et al., New England Medicine, Vol. 326, pp. 1316-1322, 1992; Nathan D. M., et al., Diabetes Care, Vol. 15, pp. 270-275, 1992).

Kindly replace the paragraph beginning at page 8, line 5, with the following:

Figure 15 is a drawing that shows an analytical pattern at each purification step in the cleavage process of GLP-1 (7-37) from RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) by Kex2 protease. In the figure A indicates a ~~[reverse=phase]~~ reverse-phase pool before cleavage with Kex2 protease, B after cleavage with the Kex2 protease, and C after PorosR2, and 1 indicates RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and 2 indicates GLP-1 (7-37).

Kindly replace the paragraph beginning at page 8, line 12, with the following:

Figure 16 is a drawing that shows the pH dependency of the amidation reaction of RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25).

Kindly replace the paragraph beginning at page 8, line 14, with the following:

Figure 17 is a drawing that shows a time course of the conversion of RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) to RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) in an amidation reaction as measured by an ion exchange HPLC, wherein 1 indicates RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and 2 indicates RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25). Absorbance was measured at 280 nm.

Kindly replace the paragraph beginning at page 23, line 15, with the following:

RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) obtained as mentioned above was subjected to an amidation modification reaction using an amidation enzyme (B. B. R. C., Vol. 150, pp. 1275-1281, 1988, EP299790A, and the like). Under the reaction condition in Examples described below, no aggregation or gelling of RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) as the enzyme substrate and the reaction product, amidated GLP-1 (7-36)NH₂ (referred to hereinafter as RHHGP-1) [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) having a helper peptide added thereto occurs, and RHHGP-1 [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) could be produced at a high reaction rate of 98% (a recovery of 95%). These results demonstrated the usefulness of the helper peptide in the amidation enzymatic reaction conducted with a substrate of RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25).

Kindly replace the paragraph beginning at page 24, line 12, with the following:

One object of the present invention on the production of GLP-1 (7-36)NH₂ is to relieve aggregation and increase solubility at the time of reaction of modification enzymes such as an amidation enzyme described above through a peptide comprising GLP-1(7-37) having a helper peptide added thereto. In order to further investigate the usefulness of the present invention, RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25), RHHGP-1 [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25), GLP-1 (7-37), and GLP-1(7-36)NH₂ were purified and the pH

dependency of the solubility of each peptide was examined. As a result, GLP-1(7-37) was shown to have a low solubility in the range of pH 5.0 to pH 7.0 as expected. On the other hand, RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) had a high solubility in the range of pH 5.0 to pH 7.0 as expected. On the other hand, RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) had a high solubility in the range of from pH 4.0 to about pH 6.0. The result confirmed that the substitution of RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) having a helper peptide for GLP-1(7-37) as an enzyme substrate is useful since the amidation enzyme reaction is carried out in the weak acid region.

Kindly replace the paragraph beginning at page 24, line 30, with the following:

In the experiment that investigated the pH dependency of solubility of each peptide, RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) have shown a sudden reduction in solubility at about pH 6.0 and about pH 6.4, respectively. GLP-1(7-36)NH₂ formed precipitates or microcrystals with time. Thus, it is estimated that substances capable of increasing the solubility of RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) in the neutral to weak alkali region and substances capable of increasing the solubility of the peptide of interest in the weak acid to weak alkali region would be very useful in the production process.

Kindly replace the paragraph beginning at page 30, line 27, with the following:

The obtained suspension of the inclusion body was diluted to give an OD660 value of 1000. From the liquid a 1000 ml portion was taken, to which 250 ml of 1M Tris-HCl for which pH has not been adjusted, 10 ml of 0.5 M EDTA (pH 8.0), and 1200 g of powdered urea was added, and then deionized water was added thereto to give a final volume of 5000 ml. Then HCL was used to adjust pH at 7.5 and the liquid was heated to 37° C for 2 hours. This procedure initialed the action of E. coli OmpT protease present in the inclusion body, and GP97ompPR was cleaved and RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) was released. Figure 10 is the result of the RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) that was excised from GP97ompPR and then was analyzed with reverse phase HPLC. The analysis used a YMC PROTEIN-PR column, a 10% acetonitrile solution containing 0.1% trifluoroacetic acid as solution A and a 70% acetonitrile solution containing 0.085% trifluoroacetic acid as solution B, with a flow rate of 1 ml/min and a linear gradient of solution B from 44% to 70% in 13 minutes. This procedure cleaved 85% of GP97ompPR and at the end of the reaction a peak corresponding to RHHGP[G] [~~SEQUENCE ID NO:25~~] (SEQUENCE ID NO:25) was obtained (Figure 21 A).

Kindly replace the paragraph beginning at page 33, line 14, with the following:

By using RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) as an intermediate, GLP-1(7-37) having a purity of 99% was obtained at ease and at a high yield. Since HPLC is not used in the purification of the present invention, it is, needless to say, easy to scale up to an industrial scale.

Kindly replace the paragraph beginning at page 33, line 26, with the following:

RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) obtained in Example 4 was converted to RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) using an amidation enzyme. In order to determine the reaction condition for the case wherein RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) was used as a substrate, optimization was carried out for pH, temperature, the concentration of copper sulfate, catalase concentration, substrate concentration, L-ascorbic acid concentration, and the concentration of the amidation enzyme in a reaction volume of 0.5 ml. Separation of RHHGP[G] ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and RHHGP-1 ~~{SEQUENCE ID NO:25}~~ (SEQUENCE ID NO:25) and analysis thereof were carried out using an ion exchange HPLC column (Poros S/H, Perceptive Biosystems) in the presence of 30 mM Britton-Robinson buffer (referred to hereinafter as the BR buffer) excluding barbital at a pH gradient elution (6.0 to 9.0).